

BEST AVAILABLE COPY

- PCT / CA 97 / 00969

06 FEBRUARY 1998 (06.02.98)  
09/331127

REC'D 10 MAR 1998

WIPO

PCT



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 14, 1998

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 08/845,546

FILING DATE: April 24, 1997

PRIORITY DOCUMENT



By Authority of the  
COMMISSIONER OF PATENTS AND TRADEMARKS

*T. Lawrence*

T. LAWRENCE  
Certifying Officer

A/no fee

## CLONED GLUCAGON-LIKE PEPTIDE 2 RECEPTORS

This is a continuation-in-part application of United States Serial No. 08/767,224, filed December 13, 1996.

### FIELD OF THE INVENTION

The invention is in the field of molecular biology. It relates, more particularly, to cloned glucagon-like peptide 2 receptors and their use in drug screening and related applications.

### BACKGROUND TO THE INVENTION

Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide, which is expressed in a tissue determined fashion from the pleiotrophic glucagon gene and is highly related in terms of amino acid sequence to glucagon and Glucagon-like peptide-1 (GLP-1). Mammalian forms of GLP-2 are highly conserved: for example, the human and degu (a south American rodent) forms differ by one and three amino acids respectively from rat GLP-2. Recently it was demonstrated that GLP-2 is an intestinotrophic peptide hormone; when given exogenously, GLP-2 can produce a marked increase in the proliferation of small intestinal epithelium of the test mice (Drucker et al, (1996) PNAS, 93:7911-7961). More recently, GLP-2 has been shown to increase D-Glucose maximal transport rate across the intestinal basolateral membrane. (Cheeseman and Tseng: American Journal of Physiology (1996) 271:G477-G482)

To accelerate research into gastrointestinal biology and development of drugs useful in the treatment of gastrointestinal disorders, it would be useful to provide the receptor through which the effects of GLP-2 are mediated.

### SUMMARY OF THE INVENTION

The GLP-2 receptor has now been cloned and characterized. Accordingly, the present invention provides an isolated polynucleotide encoding a mammalian GLP-2 receptor, including the rat and human forms. In aspects of the invention, polynucleotide coding for the GLP-2 receptor is utilized for expression to obtain functional receptor protein

and for further gene cloning to identify structurally related receptor proteins. In related aspects of the invention, anti-sense versions of GLP-2 receptor-encoding polynucleotides and fragments thereof are obtained and utilized to regulate GLP-2 receptor expression.

5 In another of its aspects, the invention provides GLP-2 receptor as a product of recombinant production in a cellular host. In related aspects, there are provided recombinant host cells that express GLP-2 receptor, as well as receptor-bearing membranes derived from such cells, and expression constructs in which polynucleotide coding for the GLP-2 receptor is linked to expression controls functional in the selected host cell.

10 In another of its aspects, the GLP-2 receptor is utilized in a chemicals screening program to identify GLP-2 receptor ligands. This method comprises the steps of incubating the candidate ligand with an GLP-2 receptor-producing cell of the present invention, or with a membrane preparation derived therefrom, and then assessing the interaction by determining receptor/candidate ligand binding.

15 In another of its aspects, the invention provides antibodies directed to the GLP-2 receptor, for use for example in diagnostic procedures.

20 The invention is further described with reference to the following drawings in which:

#### BRIEF REFERENCE TO THE DRAWINGS

25 Figure 1 discloses a cDNA sequence (SEQ ID NO: 1), nucleotides 17 to 1546 of which encode the rat GLP-2 receptor.

Figure 2 discloses the amino acid sequence of the expression product (SEQ ID NO: 2) from the cDNA of Figure 1.

30 Figure 3 illustrates the relative binding affinities of a GLP-2 peptide and a GLP-1 peptide for the receptor encoded by SEQ ID NO: 1.

Figure 4 discloses a cDNA sequence of 667 nucleotides (SEQ ID NO: 9) which encodes 222 amino acids (SEQ ID NO: 10) of the human GLP-2 receptor.

Figure 5 discloses the amino acid sequence (SEQ ID NO: 10) expressed from the cDNA of Figure 4.

Figure 6 discloses a cDNA sequence (SEQ ID NO: 11), nucleotides 121-1779 of which encode the human GLP-2 receptor.

Figure 7 discloses the amino acid sequence of the expression product (SEQ ID NO: 12) from the cDNA of Figure 6.

Figure 8 illustrates the relative binding affinities of GLP-2 peptide and GLP-1 peptide for the receptor encoded by SEQ ID NO: 11.

#### DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

The invention relates in one respect to polynucleotides that code for GLP-2 receptors. Such polynucleotides may be in the form of RNA, or in the form of DNA including cDNA, genomic DNA and synthetic DNA. The GLP-2 receptors are characterized by structural features common to the G-protein coupled receptor class, including seven transmembrane regions, and by the functional properties of binding GLP-2 peptide selectively relative to GLP-1 peptide and, when expressed functionally in a host cell, of responding to GLP-2 binding by signal transduction.

The activity of a G-protein coupled receptor such as a GLP-2 receptor can be measured using any of a variety of appropriate functional assays in which activation of the receptor results in an observable change in the level of some second messenger system, such as adenylate cyclase, calcium mobilization, inositol phospholipid hydrolysis or guanylyl cyclase.

In one embodiment of the invention, the GLP-2 receptor is encoded by the polynucleotide sequence of SEQ ID NO: 1. This particular GLP-2 receptor-encoding polynucleotide, also referred to as the WBR gene, is a cDNA of rat origin. The expression product of this polynucleotide incorporates the mature form of the GLP-2 receptor, and may incorporate a secretion signal that is removed before membrane integration of the mature GLP-2 receptor product. The expressed GLP-2 receptor product (Figure 2, SEQ ID NO: 2) is characterized structurally as a single 509 amino acid polypeptide chain having a predicted molecular weight of 59kDa. With respect to structural domains of this GLP-2 receptor, hydropathy analysis and sequence alignment with related members of this sub-family of G protein coupled receptors indicates seven putative transmembrane domains, one spanning residues 140-162 inclusive (TM I), another spanning residues 170-189 (TM II), a third spanning residues 221-244 (TM III), a fourth spanning residues 259-280 (TM IV), a fifth spanning 298-321 (TM V), a sixth spanning 345-364 (TM VI) and a seventh spanning 381-400 (TM VII). Based on this assignment, it is likely that this GLP-2 receptor, in its natural membrane-bound form, consists of an N-terminal extracellular domain, followed by a hydrophobic region containing seven transmembrane domains and an intracellular 401-509 amino acid C-terminal domain. The protein exhibits the highest degree of homology to the rat GLP-1 receptor with 38% identity at the amino acid level. Yet another aspect of the invention is amino acid sequences corresponding to any of the above domains, and nucleotide sequences which encode these amino acid sequences.

In a related embodiment, the cDNA is of human origin (Figure 3, SEQ ID NO: 9) and encodes a protein having the amino acids of SEQ ID NO: 10. This nucleic acid was isolated using the rat cDNA sequence as described generally directly below and in detail in

Example 3. In a further related embodiment of the invention, the cDNA is of human origin (SEQ ID NO: 11) and encodes the full length human GLP-2 receptor having the amino acid sequence of SEQ ID NO: 12. The human GLP-2 receptor precursor product (Figure 7, SEQ ID NO: 12) is characterized structurally as a single 553 amino acid polypeptide chain having a predicted molecular weight of 72 kDa. With respect to structural domains of this GLP-2 receptor, hydropathy analysis and sequence alignment with related members of this sub-family of G protein coupled receptors indicates seven putative transmembrane domains,

one spanning residues 181-203 inclusive (TM I), another spanning residues 211-230 (TM II), a third spanning residues 262-285 (TM III), a fourth spanning residues 300-321 (TM IV), a fifth spanning residues 339-362 (TM V), a sixth spanning residues 386-405 (TM VI), and a seventh spanning residues 422-441 (TM VII). Based on this assignment, it is likely that this GLP-2 receptor, in its natural membrane-bound form, consists of an N-terminal extracellular domain, followed by a hydrophobic region containing seven transmembrane domains interspersed with six short hydrophilic domains, and an intracellular domain, which is predicted to span residues 442-553. A second form of this GLP-2 receptor encompassed by the invention has a translational start site at the methionine codon at position 26 of the amino acid sequence presented in Figure 7 SEQ ID No. 12. The resulting 528 amino acid polypeptide chain also consists of an extracellular domain, seven transmembrane domains, and an intracellular domain, and is at least 95% identical in sequence to residues 26-553 of the sequence presented in Figure 7, SEQ ID NO: 12.

In another embodiment, the invention provides GLP-2 receptor polynucleotide sequences as a tool useful to identify structurally related polynucleotides. At low stringency hybridization conditions, for instance, polynucleotide libraries can be probed to identify genes that are at least about 40% homologous to the GLP-2 receptor gene. To facilitate isolation of WBR gene homologs that are also GLP-2 receptor-encoding, stringency conditions are desirably enhanced to identify homologs having at least 80% sequence identity at the polynucleotide level to WBR. More desirably the WBR gene homologs are 90% identical, and most desirably they have at least 95% sequence identity when compared to WBR. Preferably, the isolated WBR homologs are characterized in that (1) they can be amplified using the PCR primers of SEQ ID NO: 3 and SEQ ID NO: 4 and (2) they bind to the probe of SEQ ID NO: 5.

In a related embodiment the cDNA sequence of the human GLP-2 receptor can be used in appropriately labeled form for diagnosis of conditions associated with aberrant expression of the GLP-2 receptor.

264240-9454880  
5 In order to isolate GLP-2 receptor encoding homologs of the WBR gene, it is desirable but not necessary to use libraries of fetal or mature hypothalamal, jejunal, hindbrain or stomach tissue obtained from the vertebrate species targeted for receptor isolation. The invention accordingly includes not only the WBR but structural homologs thereof and particularly those that code for proteins having GLP-2 receptor properties. As exemplified hereinbelow, using the WBR gene as a starting material the human homolog of the rat GLP-2 receptor has now been isolated. Thus, the invention provides polynucleotides that encode GLP-2 receptors, including rat GLP-2 receptor and vertebrate homologs, particularly mammalian homologs thereof including human homologs thereof, as well as  
10 synthetic variants of these.

15 It will be appreciated that such homologs can also be identified in libraries by screening with fragments of the WBR gene, which incorporate at least 15 nucleotides, and preferably at least 25 nucleotides. With reference to SEQ ID NO: 1 and the nucleotide numbering appearing thereon, such nucleotide fragments include those corresponding in sequence to the extracellular GLP-2 binding domain, and the transmembrane regions.

20 Technically, the identification of WBR-related genes can be achieved by applying standard hybridization or amplification techniques to a tissue-derived polynucleotide library. A wide variety of such libraries are commercially available. Where construction of a cDNA library is necessary, established techniques are applied. For example, isolation of such a WBR homolog typically will entail extraction of total messenger RNA from a fresh source of tissue, such as hypothalamal, jejunal, stomach or hindbrain tissue, preferably hypothalamal tissue, followed by conversion of message to cDNA and formation of a library in for  
25 example a bacterial plasmid, more typically a bacteriophage. Such bacteriophage harboring fragments of the DNA are typically grown by plating on a lawn of susceptible E. coli bacteria, such that individual phage plaques or colonies can be isolated. The DNA carried by the phage colony is then typically immobilized on a nitro-cellulose or nylon-based hybridization membrane, and then hybridized, under carefully controlled conditions, to a  
30 radioactively (or otherwise) labelled probe sequence to identify the particular phage colony carrying the fragment of DNA of particular interest, in this case a WBR homolog. The

phage carrying the particular gene of interest is then purified away from all other phages from the library, in order that the foreign gene may be more easily characterized. Typically, the gene or a portion thereof is subcloned into a plasmidic vector for convenience, especially with respect to the full determination of its DNA sequence.

As an alternative to obtaining GLP-2 encoding DNA directly as a DNA insert from an available or a constructed cDNA library, in light of the present disclosure it can be synthesized de novo using established techniques of gene synthesis. Because of the length of the GLP-2 receptor-encoding DNAs of SEQ ID NO: 1, SEQ ID NO: 9 and SEQ ID NO: 11, application of automated synthesis may require staged gene construction, in which regions of the gene up to about 300 nucleotides in length are synthesized individually and then ligated in correct succession for final assembly. Individually synthesized gene regions can be amplified by PCR. The application of automated synthesis may typically be applied by synthesizing specific regions or fragments of the gene and ligating them, usually via designed overlaps, in correct succession to form the final gene sequence. In this case, the longer the oligonucleotide building blocks, the fewer will be the ligations needed, resulting in greater ease of assembly.

The application of automated gene synthesis techniques provides an opportunity for generating sequence variants of the naturally occurring GLP-2 receptor gene. It will be appreciated, for example, that polynucleotides coding for the GLP-2 receptor herein described can be generated by substituting synonymous codons for those represented in the naturally occurring polynucleotide sequences herein provided. In addition, polynucleotides coding for synthetic variants of the GLP-2 receptor herein provided can be generated which incorporate from 1 to 20, e.g., from 1 to 5, amino acid substitutions, or deletions or additions. Since it will be desirable typically to retain the natural ligand binding profile of the receptor for screening purposes, it is desirable to limit amino acid substitutions, for example to the so-called conservative replacements in which amino acids of similar charge are substituted, and to limit substitutions to those sites less critical for receptor activity. For example, substitution of nucleotides "G" and "A" for nucleotides "A" and "G" respectively at positions 374 and 375 of the human cDNA sequence of SEQ ID NO: 11; resulting in the



replacement of the naturally occurring arginine residue at position 85 of SEQ ID NO: 12 with a glutamic acid residue, provides a functional receptor. This functional receptor is referred to herein as the cloned residue 85 variant human GLP-2 receptor.

5 Having obtained GLP-2 receptor encoding polynucleotide, GLP-2 receptor can be produced in a number of ways, including in vitro transcription and via incorporation of the DNA into a suitable expression vector and expression in the appropriate host, for example a bacteria such as E.coli, yeast or a mammalian cell. A variety of gene expression systems have been adapted for use with these hosts and are now commercially available, and any one of these systems can be selected to drive expression of the GLP-2 receptor-encoding DNA. Expression vectors may be selected to provide transformed cell lines that express the receptor-encoding DNA either transiently or in a stable manner. For transient expression, host cells are typically transformed with an expression vector harboring an origin of replication functional in a mammalian cell. For stable expression, such replication origins are unnecessary, but the vectors will typically harbor a gene coding for a product that confers on the transformants a survival advantage, to enable their selection such as a gene coding for neomycin resistance in which case the transformants are plated in medium supplemented with neomycin.

20 These expression systems, available typically but not exclusively in the form of plasmidic vectors, incorporate expression cassettes the functional components of which include DNA constituting expression controlling sequences, which are host-recognized and enable expression of the receptor-encoding DNA when linked 5' thereof. The systems further incorporate DNA sequences which terminate expression when linked 3' of the receptor-encoding region. Thus, for expression in the selected mammalian cell host, there is generated a recombinant DNA expression construct in which the receptor-encoding DNA is linked with expression controlling DNA sequences recognized by the host, and which include a region 5' of the receptor-encoding DNA to drive expression, and a 3' region to terminate expression.

Included among the various recombinant DNA expression systems that can be used to achieve mammalian cell expression of the receptor-encoding DNA are those that exploit promoters of viruses that infect mammalian cells, such as the promoter from the cytomegalovirus (CMV), the Rous sarcoma virus (RSV), simian virus (SV40), murine mammary tumor virus (MMTV) and others. Also useful to drive expression are promoters such as the LTR of retroviruses, insect cell promoters such as those regulated by temperature, and isolated from *Drosophila*, as well as mammalian gene promoters such as those regulated by heavy metals i.e. the metallothionein gene promoter, and other steroid-inducible promoters.

In another of its aspects, the invention provides cells or membranes derived therefrom which are adapted by genetic alteration for use, for example, in identifying GLP-2 receptor ligands. In preferred embodiments, such cells are adapted genetically by the insertion of polynucleotide coding for a GLP-2 receptor. In particularly preferred embodiments, such cells incorporate a recombinant DNA molecule, e.g. an expression construct/vector, in which DNA coding for the GLP-2 receptor and expression controlling elements functional in the host are linked operably to drive expression of the DNA. For incorporation of receptor into cell plasma membranes, the vector can be designed to provide a suitable heterologous signal peptide sequence if the naturally occurring signal peptide is not encoded within the receptor DNA.

Suitable GLP-2 producing cells include the Chinese hamster ovary (CHO) cells for example of K1 lineage (ATCC CCL 61) including the Pro5 variant (ATCC CRL 1281); the fibroblast-like cells derived from SV40-transformed African Green monkey kidney of the CV-1 lineage (ATCC CCL 70), of the COS-1 lineage (ATCC CRL 1650) and of the COS-7 lineage (ATCC CRL 1651); murine L-cells, murine 3T3 cells (ATCC CRL 1658), murine C127 cells, human embryonic kidney cells of the 293 lineage (ATCC CRL 1573), human carcinoma cells including those of the HeLa lineage (ATCC CCL 2), and neuroblastoma cells of the lines IMR-32 (ATCC CCL 127), SK-N-MC (ATCC HTB 10) and SK-N-SH (ATCC HTB 11).

For use in ligand screening assays, cell lines expressing the receptor-encoding DNA can be stored frozen for later use. Such assays may be performed either with intact cells, or with membrane preparations derived from such cells. The membrane preparations typically provide a more convenient substrate for the ligand binding experiments, and are therefore preferred as binding substrates. To prepare membrane preparations for screening purpose, i.e., ligand binding experiments, frozen intact cells are homogenized while in cold water suspension and a membrane pellet is collected after centrifugation. The pellet is then washed in cold water, and dialyzed to remove any endogenous GLP-2 receptor ligands that would otherwise compete for binding in the assays. The dialyzed membranes may then be used as such, or after storage in lyophilized form, in the ligand binding assays.

The binding of a candidate ligand to a selected GLP-2 receptor of the invention is performed typically using a predetermined amount of cell-derived membrane (measured for example by protein determination), generally from about 25ug to 100ug. Generally, competitive binding assays will be useful to evaluate the affinity of a test compound relative to GLP-2. This competitive binding assay is performed by incubating the membrane preparation with radiolabelled GLP-2 peptide, for example [ $^3$ H]-GLP-2 or a radioiodinated GLP-2 analog, in the presence of unlabelled test compound added at varying concentrations. Following incubation, either displaced or bound radiolabelled GLP-2 can be recovered and measured, to determine the relative binding affinities of the test compound and GLP-2 for the GLP-2 receptor used as substrate. In this way, the affinities of various compounds for the GLP-2 receptor can be measured.

Alternatively, intact, fresh cells, harvested about two days following after transient transfection or after about the same period following fresh plating of stably transfected cells, can be used for ligand binding assays by the same methods as used for membrane preparations. In this case, the cells must be harvested by more gentle centrifugation so as not to damage them, and all washing must be done in a buffered medium.

As an alternative to using cells that express receptor-encoding DNA, ligand characterization may also be performed using cells for example *Xenopus* oocytes, that yield functional

membrane-bound receptor following introduction of messenger RNA coding for the GLP-2 receptor. In this case, the GLP-2 receptor gene of the invention is typically subcloned into a plasmidic vector such that the introduced gene may be easily transcribed into RNA via an adjacent RNA transcription promoter supplied by the plasmidic vector, for example the T3 or T7 bacteriophage promoters. RNA is then transcribed from the inserted gene in vitro, and can then be injected into *Xenopus* oocytes. Each oocyte is a single cell, but is large enough to be penetrated by a fine-tipped microneedle without causing irreparable damage. Following the injection of nL volumes of an RNA solution, the oocytes are left to incubate for up to several days, whereupon the oocytes are tested for the ability to respond to a particular ligand molecule supplied in a bathing solution.

Candidate GLP-2 receptor ligands can vary widely in structure, and include proteins which are highly related to GLP-2 itself in terms of amino acid sequence. For instance, the peptides disclosed in co-pending United States patent applications 08/422,540 and 08/631,273, incorporated herein by reference, may usefully be screened for GLP-2 receptor binding activity. Furthermore, the advent of high throughput screening makes feasible the screening of a chemical library containing hundreds or thousands of test compounds for GLP-2 receptor binding activity.

In addition to using the receptor-encoding DNA to construct cell lines useful for ligand screening, expression of the DNA can according to another aspect of the invention be performed to produce fragments of the receptor in soluble form, for structure investigation, to raise antibodies and for other experimental uses. It is expected that the portion of the GLP-2 receptor responsible for binding a ligand molecule resides on the outside of the cell, i.e., is extracellular. It is therefore desirable in the first instance to facilitate the characterization of the receptor-ligand interaction by providing this extracellular ligand-binding domain in quantity and in isolated form, i.e., free from the remainder of the receptor.

To accomplish this, the full-length GLP-2 receptor-encoding DNA may be modified by site-directed mutagenesis, so as to introduce a translational stop codon into the extracellular

N-terminal region, immediately before the sequence encoding the first transmembrane domain (TM1), i.e., before residue 140 of SEQ ID NO: 2 and before residue 181 of SEQ ID NO: 12. Since there will no longer be produced any transmembrane domain(s) to "anchor" the receptor into the membrane, expression of the modified gene will result in the secretion, in soluble form, of only the extracellular ligand-binding domain. Standard ligand-binding assays may then be performed to ascertain the degree of binding of a candidate compound to the extracellular domain so produced. It may of course be necessary, using site-directed mutagenesis, to produce several different versions of the extracellular regions, in order to optimize the degree of ligand binding to the isolated domains.

It will be appreciated that the production of such extracellular ligand binding domains may be accomplished in a variety of host cells. Mammalian cells such as CHO cells may be used for this purpose, the expression typically being driven by an expression promoter capable of high-level expression, for example the CMV (cytomegalovirus) promoter. Alternately, non-mammalian cells, such as insect Sf 9 (*Spodoptera frugiperda*) cells may be used, with the expression typically being driven by expression promoters of the baculovirus, for example the strong, late polyhedrin protein promoter. Filamentous fungal expression systems may also be used to secrete large quantities of such extracellular domains of the GLP-2 receptor. *Aspergillus nidulans*, for example, with the expression being driven by the *alcA* promoter, would constitute such an acceptable system. In addition to such expression hosts, it will be further appreciated that any prokaryotic or other eukaryotic expression system capable of expressing heterologous genes or gene fragments, whether intracellularly or extracellularly would be similarly acceptable.

The availability of isolated extracellular ligand-binding domains of the receptor protein makes it feasible to determine the 3-dimensional structures of these ligand-binding regions, with or without a candidate ligand complexed thereto, by a combination of X-ray crystallographic and advanced 2D-NMR techniques. In this way, additional new candidate compounds, predicted to have the required interactions with the 3-dimensional receptor structure, can be specifically designed and tested.

084546-04497  
264240-9454880

5 With large domains, crystallography is the method of choice for structure determination of both the domain in isolation, and of the co-complex with the natural ligand (or an appropriate antagonist or agonist molecule). If a particular domain can be made small enough, for example approximately 100-130 amino acids in length, then the powerful technique of 2-D NMR can also be applied to structure determination. This enables not only the determination of the domain structure, but also provides dynamic information about the drug-receptor interaction.

10 For use particularly in detecting the presence and/ or location, for example in intestinal tissue, the present invention also provides, in another of its aspects, labelled antibody to a GLP-2 receptor. To raise such antibodies, there may be used as immunogen either the intact, soluble receptor or an immunogenic fragment thereof, produced in a microbial or mammalian cell host as described above or by standard peptide synthesis techniques. Regions of the GLP-2 receptor particularly suitable for use as immunogenic fragments include those corresponding in sequence to an extracellular region of the receptor, or a portion of the extracellular region, such as peptides consisting of 10 or more amino acids of the 401-509 region of SEQ ID NO: 2. With regard to the human GLP-2 receptor (SEQ ID NO: 12), peptides comprising the mature extracellular domain (residues 65-180); intracellular loop 3 (residues 363-385) and the intracellular C-terminal domain (residues 442-533) may be usefully employed as immunogens for the production of antibodies to the human GLP-2 receptor.

25 Antibodies to the desired GLP-2 receptor or fragment immunogen are available, for polyclonal antibody production, from the blood of an animal that has been immunized with the immunogen. Alternatively, for monoclonal antibody production, immunocytes such as splenocytes can be recovered from the immunized animal and fused, using hybridoma technology, to a myeloma cells. The fusion products are then screened by culturing in a selection medium, and cells producing antibody are recovered for continuous growth, and antibody recovery. Recovered antibody can then be coupled covalently to a detectable label, such as a radiolabel, enzyme label, luminescent label or the like, using linker technology established for this purpose.

30

084546-042497  
264240-9454880

5  
10  
15  
20  
25  
30

Animal model systems which elucidate the physiological and behavioral roles of the GLP-2 receptor are produced by creating transgenic animals in which the activity of the GLP-2 receptor is either increased or decreased, or the amino acid sequence of the expressed GLP-2 receptor is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a GLP-2 receptor, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these GLP-2 receptor sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native GLP-2 receptors but does express, for example, an inserted mutant GLP-2 receptor, which has replaced the native GLP-2 receptor in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added GLP-2 receptors, resulting in overexpression of the GLP-2 receptors.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium. DNA or cDNA encoding a GLP-2 receptor is cesium chloride purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the transgene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a piper puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a

pseudopregnant mouse ( a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only methods for inserting DNA into the egg cell, and is used here only for exemplary purposes.

The invention having been described above, may be better understood by referring to the following examples. The following examples are offered for the purpose of illustrating the invention and should not be interpreted as a limitation of the invention.

#### EXAMPLE 1

##### Isolation of the GLP-2 receptor

##### PCR-assisted cloning of partial rat and mouse GLP-2 receptor cDNAs

Rat Neonate Intestine cDNA library (Stratagene, La Jolla, CA; Cat. 936508) and Mouse Jejunum first strand cDNA was prepared. Degenerate primers M-2F/S (SEQ ID NO: 3) and M-7R/S (SEQ ID NO: 4) were used to amplify a partial fragment of the rat GLP-2 receptor from the Rat Neonate Intestine cDNA library and of the mouse GLP-2 receptor from Mouse Jejunum template. The protocol is described below:

##### Degenerate PCR :

6  $\mu$ l 10x VENT buffer from New England Biolabs

6  $\mu$ l 2.5 $\mu$ M each stock dATP, dCTP, dGTP and dTTP

4  $\mu$ l rat neonate intestine cDNA (1:10 dilution)

3  $\mu$ l 25 $\mu$ M primer [5'-TTTTTCTAGAASRTSATSTACACNGTSGGCTAC-3'] (SEQ ID NO: 3)

3  $\mu$ l 25 $\mu$ M primer [5'-TTTTCTCGAGCCARCARCCASSWRTARTTGGC-3'] (SEQ ID NO: 4)

2  $\mu$ l (10 units) Amplitaq DNA polymerase (Perkin Elmer)

36  $\mu$ l ddH<sub>2</sub>O.

Reaction conditions: 35 cycles at 94°C, 2°min.; 94°C, 1 min.; 53°C, 30 sec.; 72°C, 1 min.



The predominant PCR product was a 303 base pair (bp) DNA fragment. 30  $\mu$ l samples of the above PCR were purified using the QIAGEN PCR purification kit and eluted in 30  $\mu$ l ddH<sub>2</sub>O.

5 Reamplification PCR:

6  $\mu$ l 10x VENT buffer from New England Biolabs

6  $\mu$ l 2.5 $\mu$ M each stock dATP, dCTP, dGTP and dTTP

4  $\mu$ l above purified PCR template

3  $\mu$ l 25 $\mu$ M primer [5'-TTTTTCTAGAASRTSATSTACACNGTSGGCTAC-3']

10 3  $\mu$ l 25 $\mu$ M primer [5'-TTTTCTCGAGCCARCARCCASSWRTARTTGGC-3']

2  $\mu$ l (10 units) Amplitaq DNA polymerase (Perkin Elmer)

36  $\mu$ l ddH<sub>2</sub>O

Reaction conditions: 31 cycles at 94°C, 2 min.; 94°C, 1 min.; 53°C, 30 sec.; 72°C, 1 min.

15 The predominant product at 303 base pair (bp) was cut out and purified using QIAGEN QIAquick gel purification protocol into 30  $\mu$ l ddH<sub>2</sub>O.

Next, double digest (Xba I and Xho I) was done on the entire reamplified PCR reaction as follows:

20 28  $\mu$ l DNA

16  $\mu$ l 10X One-Phor-All buffer (Pharmacia)

2  $\mu$ l (40 units) Xba I enzyme (Pharmacia)

2  $\mu$ l (40 units) Xho I enzyme (Pharmacia)

30  $\mu$ l ddH<sub>2</sub>O.

25

The samples were digested 4 hours in 37°C water block heater, brought up to 100  $\mu$ l volume with ddH<sub>2</sub>O (sterile) and purified by (1) equal amount (100 $\mu$ l) chloroform extraction; (2) weekend precipitation with 2 volumes ethanol/10 volumes 3M sodium acetate; (3) 1x wash with 70% EtOH; and (4) resuspension in 10  $\mu$ l 1x TE (pH 8.0).

30

pBluescript clone 5HT1F#9 was next digested with Xba I and Xho I as follows:

10 µl DNA (pBluescript clone 5HT1F#9)  
5 µl 10X NEBuffer 2 (New England Biolabs)  
3 µl (1:20 dilution=3 units) Xba I (New England Biolabs)  
3 µl (1:20 dilution=3 units) Xho I (New England Biolabs)  
5 µl (10x) BSA (New England Biolabs)  
24 µl ddH<sub>2</sub>O.

The sample was digested for 3 hours in 37 C water block heater, heat-inactivated at 65 C for 20 min and purified using GeneCleanII kit from BIO 101. Aliquots of the PCR reactions were cloned into the above pBluescript plasmid vector using T4 DNA ligase kit (New England Biolabs) and transformed into Epicurean Coli XL-2 Blue MRF' Ultracompetent cells (Stratagene). The transformation was plated onto 2xYT + AMP plates and single colonies were picked. DNA minipreps were made using QIAGEN QIA-prep 8 miniprep kit and the sequences of the fragments were determined using ABI system. Novel sequences were identified containing a partial fragment of the rat and mouse GLP-2 receptor sequence.

Cloning of cDNA with complete GLP-2 receptor coding region was achieved as follows: First, cDNA libraries from the following three tissues were used for screening,

1. Rat Hypothalamus (RHT)
2. Rat Hind Brain (RHB)
3. Rat Duodenum and Jejunum (RDJ)

The three cDNA libraries were prepared by priming with random primer and subcloning unidirectionally into Hind III and Not I sites of pcDNA3.

Next, the three cDNA libraries were homology screened by a degenerate oligo C4-4 [5'-AACTACATCCACMKGMAYCTGTTYVYGTCBTTCATSCT-3'] (SEQ ID NO: 5) by colony lifts and filter hybridization. The following hybridization conditions were employed: 5X SSPE (1X SSPE is 0.18M NaCl, 10mM NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 10mM EDTA (pH 7.4)) and 5X Denharts solution (1% Ficoll, 1% Polyvinylpyrrolidone, 1% BSA); 25 mg/ml salmon sperm DNA.

Filters were hybridized at 50°C overnight. Then the filters were washed 2 times in 2X SSPE and 1% SDS at room temperature for 30 min, 2 times in 2X SSPE and 1% SDS at 50°C for 20 min per wash, and finally two times in 1X SSPE and 0.5% SDS. Positive clones were identified by autoradiography. A plug of 1cm<sup>2</sup> surrounding the positive clone was removed from the plate and placed in 1 ml of 2x YT +20% Glycerol, vortexed and was frozen at -80°C.

Plasmid DNA from positive plugs was prepared as follows: 100 ml of bacterial culture of each positive plug was grown on an agar plate. The bacterial cells were scraped and resuspended in 1 ml of 2xYT medium+20% Glycerol. Bacterial pellet from the 250 ml of bacterial resuspension was resuspended in 150 ml of Solution I (50mM Glucose, 10mM Tris-HCl, 1mM EDTA), lyse in Solution II (0.2M NaOH, 1% SDS), neutralized with ice cold Solution III (Potassium acetate; 4 vol. of 5M potassium acetate + 1 vol. of 10M acetic acid). After pelleting bacterial DNA, 340 ml isopropanol was added to the supernatant. This was centrifuged at max for 15 min. The pellet was resuspended in TE + 20 mg/ml RNase, incubated at 37 C for 30 minutes and precipitated with isopropanol + 0.2M potassium acetate. After centrifugation, the pellet was washed with 70% alcohol, allowed to air dry and resuspended in TE.

Plasmid DNA from 2777-clone pools of rat hypothalamus cDNA library RHT cDNA library was next exploited as follows: Two primers were designed from an area of the PCR-cloned GLP-2 receptor cDNA sequence that did not have identity to known receptors of the gene family. The two primers P23-R1 and P23-F1 amplified a 196 bp fragment only from novel clone DNA but not with GLP-1 receptor cDNA or PACAP receptor cDNA. The Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842) was used under the following conditions:

2 µl of 10x Expand™ Buffer 1

2.8 µl of 2.5mM dNTP mix

0.6 µl of primer P23-R1 [5'-TCATCTCCCTCTTCTTGGCTCTTAC-3'] (SEQ ID NO:

6)

0.6 µl of primer P23-F1 [5'-TCTGACAGATATGACATCCATCCAC-3'] (SEQ ID NO: 7)

0.3 µl of Expand PCR enzyme (1 unit)

12.7 µl water

1 µl DNA

Reaction conditions: 32 cycles at 93°C, 40 sec; 58°C, 40 sec; 68°C, 40 sec

DNAs from each positive plug or pool of 2777-clone pools were amplified with specific primers P23-F1 and P23-R1 under the conditions specified above. Out of 1057 C4-4 hybridization-positive plugs and 884 2777-clone pools only five template sources amplified a 196 bp PCR product. These were: (1) Plug 334, (2) Plug 780, (3) RHT pool 233, (4) RHT pool 440, and (5) RHT pool 587.

Amplification of GLP-2R cDNA from the five positive templates was then performed. By using one specific primer (P23-R1 or P23-F1) and one primer based on pcDNA3 vector (Invitrogen) sequence (830F or 1186R), the GLP-2R cDNA insert was directly amplified from clonally impure plugs or 2777-clone pools. The sequences of the vector primers were as follows:

830F: [5'-AACCCACTGCTTAC-3']

1186R: [5'-CCCAGAATAGAATGACACC-3']

The PCR was done under the following conditions using Expand™ PCR system from Boehringer Mannheim (Catalogue no. 1681-842).

2 µl of 10x Expand™ Buffer 1

2.8 µl of 2.5mM dNTP mix

0.6 µl of Primer 1

0.6 µl of Primer 2

0.3 µl of Enzyme (1 unit)

12.7 µl water

1 µl DNA

Reaction conditions: 32 cycles at 93°C, 45 sec; 50°C, 45 sec; 68°C, 1 min.

264240-94554380  
5 The most prominent band was reamplified, purified and sequenced. Based on the amplified sequence obtained, additional primers were designed and new sequencing carried out. In this manner the complete sequences of the GLP-2R cDNA inserts in all five sources of clones were determined. Sequence analysis showed that only pool RHT 440 and pool RHT 587 contain clones with complete coding sequence of GLP-2R and that the two clones were identical (derived from the same cDNA clone).

10 Because of difficulty in clonally purifying the GLP-2 receptor cDNA clone from the RHT 440 or RHT 587 cDNA library pools, the cDNA was amplified and recloned into pcDNA3. Based on the sequence obtained from RHT 440 and RHT 587, two primers were designed adjacent to the 5' and 3' ends of the coding region.

WBR-C5: [5'-CAGGGGCCGGTACCTCTCCACTCC-3']

WBR-C3: [5'-TTGGGTCCTCGAGTGGCCAAGCTGCG-3']

15 The two primers were used to amplify a DNA fragment of approximately 1525 bp fragment under the following PCR conditions using Expand™ PCR system from Boehringer Mannheim (Catalogue no. 1681-842).

10 µl of 10x Expand™ PCR Buffer 1

14 µl of 2.5mM dNTP mix

20 3.0 µl of Primer 1 (10 µM)

3.0 µl of Primer 2 (10 µM)

1.5 µl of Enzyme (5 units)

63.5 µl water

5 µl DNA

25 Reaction conditions: 5 cycles ( 93°C, 1 Min; 72°C, 40s; 60°C, 45 sec; 68°C, 2 min) and 25 cycles ( 93°C, 1 min; 72°C, 1 min; 68°C, 2 min).

30 The amplified product was subcloned into Kpn I and Xho I sites of pcDNA3 vector (Invitrogen). Plasmid DNA was prepared using the method described above.

## EXAMPLE 2

### Ligand Binding Assay

Cos-1 cells were transfected as described in Analytical Biochemistry, 218:460-463(1994) with Rat clone 587 GLP-2 receptor, cloned human GLP-2 receptor, or cloned residue 85 variant human GLP-2 receptor. Rat GLP-1 (7-36) amide was used as a control peptide.

Solutions used were as follows:

RSC in RPMI 1640 (49ml RPMI + 1ml FCS + 50ul chloroquine, 100mM);

DEAE/RSC Solution: 18.4 ml RSC +1.6 ml DEAE/Dextran (10 mg/ml).

The assay procedure entailed the following:

a) 50 mg of either rat clone 587 GLP-2 receptor, or cloned human GLP-2 receptor, or cloned residue 85 variant human GLP-2 receptor was added to a 50 ml tube containing six mls. of RSC and incubated at 37°C.

b) Six ml of DEAE/RSC solution was added to each tube and incubate at 37°C for 2 min.

c) 1.5 ml of COS-1 cell suspension (5.5 millions cells) was added to each tube and incubated for 1 hr 45 min at 37°C.

d) Following incubation, the sample was spun for 5 minutes at low speed, washed with DMEM/F12 + 10% FBS twice, and the pellet resuspended in 12.5 ml DMEM/F12 + 10% FBS media.

e) One ml of cell suspension (step d) was added to each well of 6 well plates coated with poly- D-lysine (from Collaborative Biomedical), containing 3 ml of media (0.45 million cells/well).

f) Plates were incubated at 37°C for 3 days.

Treatment of Transfected Cos-1 cells with GLP-1/GLP-2 analog was done as follows:

Solutions : DMEM/F12 (SFM)+ IBMX (3-isobutyl-1-methylxanthin) 0.85mM +0.1% ascorbic acid and 10 um pargyline (all solutions purchased from Sigma). Media was prepared fresh on day of use.

Assay Procedure: The culture media of each well (transfected 6 well plates, cells) was removed, and the wells were washed once with SFM media. Then 2 ml of SFM +

IBMX media was added to each well and plates were incubated at 37°C for 10 min. Following incubation, the SFM + IBMX was removed from each well and fresh SFM + IBMX media containing GLP-1/GLP-2 (GLP-1,7-36,amide from Sigma, [Gly2]hGLP-2 from Allelix) concentration 1, 3, 10 and 30 nM were added to the appropriate wells. Plates incubated at 37°C incubator for 30 min. Following incubation, the media were removed from each well. The wells were washed once with 1ml PBS (Phosphate Buffered Saline). Each well was then treated with 1ml cold 95% ethanol: 5mM EDTA (2:1) at 4°C for 1 hr. Cells from each well then were scraped and transferred into individual eppendorf tubes. Tubes were centrifuged for 5 min at 4°C, and the supernatants were transferred to new eppendorf tubes and dried in speed vacuum. Following drying, tubes were reconstituted in 100ul of Na- Acetate and kept at 4°C, 25 µl of this solution used for cAMP Assay.

The functional assay was performed as follows: cAMP content for each extract was determined in duplicate by EIA (Enzyme ImmunoAssay) using the Amersham Biotrak cAMP EIA Kit (Amersham 225). Results of the assays, illustrated in Figure 3 and Figure 6, demonstrate the GLP-2 selectivity exhibited by the cloned rat and human receptors. In a similar assay of binding to the GLP-1 receptor, the expected pattern of binding preferentially to GLP-1 was observed.

### EXAMPLE 3

#### Isolation Of Human GLP-2 Receptor cDNA

##### Medium-stringency hybridization screening of a human hypothalamus cDNA library

One million clones from a  $\lambda$ gt10 cDNA library from human hypothalamus (Clontech; Cat. No. 1172a) were screened by plaque lifts on nitrocellulose filters (Amersham; Cat.RPN137E). The probe was prepared by random primer labeling a DNA fragment containing the complete coding region of rat GLP-2 receptor. The DNA fragment was isolated from clone 587-C1, which contains the complete coding region from SEQ ID NO: 2.

Pre-hybridization and hybridization were each carried out overnight in a hybridization solution consisting of 50% formamide, 5X SSPE, 5X Denhart's solution, 0.5% SDS and salmon sperm DNA (200 mg/ml). After hybridization the filters were washed under the following conditions (medium stringency):

- two times at room temperature in 2X SSPE and 0.01% SDS.
- two times at 42°C in 2X SSPE and 0.01% SDS.
- two times at 42°C in 0.2X SSPE and 0.01% SDS.

The filters were autoradiographed and agar plugs, each containing numerous bacteriophage plaques, were picked from regions on the plates corresponding to positive signals on the filter. From one million cDNA clones sampled in the first round screen, we identified two positive clones (HHP6-1 and HHP13). On secondary screening only HHP13 turned out positive. Several positive plaques (HHS13) from the HHP13 plate were pooled and taken for tertiary screening. Three single positive plaques from this round of screening were picked (HHT13-1, HHT13-2, HHT13-3).

PCR amplification was then used for partial sequencing of the positive clones. On a lawn of bacterial cells (E.coli C600Hfl), 10 ml of phage suspension from each clone was applied at marked spots. After 5 hr incubation at 37°C, the phage plaques were clearly visible and covered ~1 cm<sup>2</sup>. A portion of each plaque was transferred to 200 ml of water. The samples were incubated in a boiling water bath for 5 min and centrifuged at room temperature for 10 min. One milliliter of sample was used for PCR amplification with two sets of degenerate primers:

M2FS[5'-ITTTTCTAGAASRTSATSTACACNGTSGGCTAC-3'] (SEQ ID NO: 3) and M7RS[5'-TTTTCTCGAGCCARCARCCASSWRTARTTGGC-3'] (SEQ ID NO: 4); or C4-4 [5'-AACTACATCCACMKGMAYCTGTTYVYGTCBTTCATSCT-3'] (SEQ ID NO: 5) and

C9-2R [5'-TCYRNCTGSACCTCMYYRTTGASRAARCAGTA-3'] (SEQ ID NO: 8).

The Expand<sup>TM</sup> PCR system from Boehringer Mannheim (Cat. 1681-842) was used under the following conditions:

5 µl of 10x Expand<sup>TM</sup> Buffer 3



7 µl of 2.5mM dNTP mix  
1.5 µl of primer M2FS or C4-4  
1.5 µl of primer M7RS (with M2FS) or C9-2R (with C4-4)  
0.75 µl of Expand PCR enzyme (1 unit)  
5 33.25 µl water and  
1 µl DNA.

Reaction conditions were: 32 cycles at 93°C, 1 min; 50°C, 1 min; 45°C, 1 min; 68°C, 2 min.

10 M2FS and M7RS amplified a DNA fragment of about 300 bp and C4-4 and C92-R amplified a DNA fragment of about 700 bp. The PCR products were purified using the QIAGEN QIAquick PCR purification kit (Cat. 28104) and eluted in 50 µl 10 mM Tris, pH 8.0. Sequence analysis of the products revealed no differences between the  
15 templates, as expected from the fact that they represent multiple copies of a single cDNA clone (HHT13).

A number of factors indicate that this clone contains coding sequence of the human GLP-2 receptor. One factor is the degree of sequence similarity. The glucagon  
20 receptor cDNA can be used to predict the expected degree of sequence conservation found between rat and human receptors. At the nucleotide level, there is 82.6% identity within the coding regions of the rat and human glucagon receptors. At the amino acid level, there is 80.9% identity and 89.1% amino acid similarity between the glucagon receptors of the two species.

25 In the case of the human GLP-2 receptor cloned herein, the sequence of the partial human GLP-2 receptor cDNA (HHT13) is highly homologous to rat GLP-2 receptor cDNA at both the nucleotide and amino acid level. SEQ ID NO: 9 shows 87.1% identity with the rat GLP-2 receptor cDNA sequence. The predicted amino acid sequence of this  
30 cDNA region has 87.4% identity and 93.2% similarity with the predicted amino acid sequence of the rat GLP-2 receptor. The total predicted length of the rat receptor preprotein is 509 amino acids, suggesting we have determined the sequence of about

44% of the coding region of the human receptor. The comparison of the predicted partial human sequence with the predicted amino acid sequence of rat GLP-2 receptor shows beyond a reasonable doubt that this cDNA encodes the human counterpart of the rat receptor.

Further evidence supporting this conclusion comes from a comparison of the partial human GLP-2 receptor amino acid sequence with the rat GLP-2 receptor and the 3 next closest family members, shown below:

Receptor Sequence (amino acid)	Percent Identity with HHT13	Percent Similarity
GLP-2 receptor (rat)	87.4	93.2
GLP-1 receptor (rat)	50.0	74.1
Glucagon receptor (rat)	51.4	73.9
GIP receptor (rat)	50.7	70.3

These comparisons, together with the benchmark provided by sequence similarities between the rat and human glucagon receptors, provide definitive evidence that the cDNA designated HHT13 represents the human counterpart of the rat GLP-2 receptor.

The full amino acid sequence of the human GLP-2 receptor is obtained by first determining the sequence of the complete cDNA inserts in HHT13-1, HHT13-2 and HHT13-3. By using degenerate primers for PCR amplification and subsequent sequencing, we obtained sequence from only part of each insert. It is possible that these identical clones contain an insert which spans the complete coding sequence of the human GLP-2 receptor preprotein. To determine the complete sequence of the cDNA insert, the clones are grown in large quantity to prepare approximately 20 mg of each equivalent clone. The complete cDNA insert is excised by restriction with Eco RI, and subcloned into pcDNA3 (Invitrogen). Alternatively, two primers from vector sequence flanking the insert are used to amplify the complete cDNA insert using the Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842). The amplified cDNA is cut with appropriate restriction enzymes and is subcloned into pcDNA3 (Invitrogen).

If a complete coding sequence is not present in the HHT13 clones, cDNA libraries are screened for additional clones to complete the coding region of human GLP-2 receptor cDNA. Preferably human cDNA libraries (from Stratagene or Clontech) representing the following tissues are used for screening: Human hypothalamus; Human fetal brain; Human duodenum and jejunum; Human stomach; and Human fetal intestine.

Two PCR primers are designed from the sequence of human GLP-2 receptor cDNA already determined. These primers are designed such that they could not amplify any related gene family members other than the GLP-2 receptor cDNA itself. A dilution of the cDNA library stock is used to make library sub-pools such that 50,000 clones are represented in each pool. PCR is conducted with the GLP-2 receptor-specific primers to diagnose pools containing a GLP-2 receptor cDNA clone, using the Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842) under the following conditions:

2 µl of 10x Expand™ Buffer 1

2.8 µl of 2.5mM dNTP mix

0.6 µl of primer P1

0.6 µl of primer P2

0.3 µl of Expand PCR enzyme (1 unit)

12.7 µl water

1 µl of library pool containing 50,000 clones

Reaction conditions: 32 cycles at 93°C, 40 sec; 50-58°C, 40 sec; 68°C, 40 sec.

Sequence is then obtained from the complete GLP-2 receptor cDNA insert from a positive pool. By using one specific primer and one primer based on vector sequence close to the cloning site, the GLP-2 receptor cDNA insert is directly amplified from clonally impure clone pools, using the Expand™ PCR system from Boehringer Mannheim (Catalogue no. 1681-842) most suitably under the following conditions:

2 µl of 10x Expand™ Buffer 1

2.8 µl of 2.5mM dNTP mix

0.6 µl of Primer 1

0.6 µl of Primer 2

0.3 µl of Enzyme (1 unit)

12.7 µl water

1 µl of library pool stock

Reaction conditions: 32 cycles at 93°C, 45 sec; 50°C, 45 sec; 68°C, 1 min.

5 The reaction is run on a preparative agarose gel, and the most prominent band is purified and sequenced. Based on the amplified sequence obtained, additional primers are designed to obtain sequence and clones of complete coding region and clone the complete cDNA

10 5' RACE and 3' RACE are used to obtain complete coding sequence of the human GLP-2 receptor cDNA. Rapid Amplification of cDNA Ends (RACE) is a procedure routinely used for amplification of DNA sequences from first cDNA strand (easily prepared from mRNA) template between a defined internal site and either 3' or the 5' end of the mRNA. Total or mRNA from different human tissues are commercially available from Clontech. The 3' RACE System (Gibco-BRL Life Technologies; Cat. 18373-019) and 5' RACE System (Cat. 18374-058) kits are used. The manuals of these two products provide detailed protocols. In brief, protocols are as described below.

20 For the 3' RACE procedure, first strand cDNA synthesis is initiated at the poly (A) tail of mRNA using the adapter primer (provided with system) incorporating a unique sequence for universal PCR amplification of the RACE products. After synthesis of the first strand cDNA from this primer, the original mRNA template is destroyed with RNase H. Amplification is then performed using two primers: one is a gene-specific primer (which will be designed from the available partial cDNA sequence of HHT13); the other  
25 is the universal amplification primer provided with the kit. The amplified product is subcloned into a plasmid vector for sequencing.

30 For the 5' RACE System, the first strand cDNA is synthesized from mRNA using a gene-specific primer (which is based on the available partial cDNA sequence of HHT13) and SuperScript II reverse transcriptase. The original mRNA template is removed by treatment with RNase H. Unincorporated dNTPs, primer, and proteins are separated

from cDNA using spin cartridge. A homopolymeric dCTP tail is then added to the 3' end of the first strand cDNA using TdT enzyme and dCTP nucleotides. PCR amplification is performed using two primers: one is a nested, gene-specific primer designed from the available partial DNA sequence of HHT13; and the other is an "anchor primer" provided with the system. Both primers incorporate restriction sites for subcloning into plasmids and subsequent sequencing.

Sub-cloning of HHT13  $\lambda$ gt10 clones into pcDNA3, their sequencing and expression

A. Amplification of cDNA inserts with  $\lambda$ gt10 primers.

On a lawn of bacterial cells (E.coli C600Hfl), 10  $\mu$ l of phage resuspension from each clone was placed at marked spots. After 5 hr incubation at 37°C, the phage plaques were clearly visible. The surface of each plaque was transferred to 200  $\mu$ l of water. The samples were kept in boiling water bath for 5 minutes and centrifuged at room temperature for 10 minutes. 1  $\mu$ l of sample was used to amplify with a set of  $\lambda$ gt10 primers.

GT10-5KXb [5'-GGGTAGTCGGTACCTCTAGAGCAAGTTCAGCC-3']

vs

GT10-3BXh [5'-ATAACAGAGGATCCTCGAGTATTTCTTCCAG-3']

The Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842) was used under the following conditions:

5  $\mu$ l of 10x Expand™ Buffer 3

7  $\mu$ l of 2.5mM dNTP mix

1.5  $\mu$ l of primer GT10-5KXb

1.5  $\mu$ l of primer GT10-3BXh

0.75  $\mu$ l of Expand PCR enzyme (1 unit)

33.25  $\mu$ l water

1  $\mu$ l DNA

Reaction conditions: 5 cycles of 93°C-40 sec; 50°C- 1 min; 68°C-2 min and 30 cycles of 93°C-40 sec; Ramp to 68°C- 1 min; 68°C-2 min.

08845546.042497

An amplified DNA fragment of about 2200 bp long was seen on the agarose gel from all three clones. The PCR product were purified using the QIAGEN's QIAquick PCR purification kit (Cat. no. 28104) and eluted in 50 µl 10 mM Tris, pH 8.0. The templates were sequenced.

5

B. Subcloning into pcDNA3 vector.

10

The amplified and purified DNA from the three clones was restricted with Kpn I and Xho I and subcloned into pcDNA3 restricted with similar restriction enzymes. The plasmids were named pHHT13-1, pHHT13-2, and pHHT13-3. Plasmids DNAs were prepared using either crude method (alkaline treatment, bacterial DNA precipitation with 3 M KOAc, isopropanol precipitation followed by RNase treatment and second round of isopropanol precipitation) or plasmid DNA kits from Qiagen Inc. The templates prepared using Qiagen's kits were sequenced.

15

C. Functional Assay

20

Transfections were carried out with each clone, using the rat GLP-2R, 587 clone as a positive control for cAMP response to GLP-2 peptide. Methods for transfection, cell culture and cAMP assay were identical to those described for the functional assay of rat, 587 clone. Results showed that although the positive control gave good cAMP response in COS cells, none of the HHT13 clones gave any cAMP response. As confirmed by sequencing which showed a frame-shift mutation, the functional data suggested that no functional GLP-2R protein was expressed from these cDNA clones.

25

D. Comparison of DNA sequences between rat GLP-2R and HHT13 subclones.

The comparison showed a 2 bp deletion at a position corresponding to nucleotides 389-390 of the rat GLP-2R cDNA, resulting in the loss of nucleotides 374-375 of the human GLP-2R cDNA sequence presented herein.

Insertion of 2 base pairs at position 374 of the human GLP-2 receptor cDNA sequence  
and Functional Analysis of the resulting clone

PCR was used to incorporate two bp of the rat GLP-2R DNA sequence into HHT13-1 DNA at the site of the 2 bp frame-shift deletion identified relative to the rat GLP-2R coding sequence. The following primers were designed from HHT13 DNA sequence to insert two bp :

HWBR/2BPI-475F

[5'-ACAGGCATGTCTGGAAGACTTACTCAAGGAACCTTCTGGCAT-3']

HWBR/2BPI-506R

[5'-ATGCCAGAAGGTTTCCTTGAGTAAGTCTTCCAGACATGCCTGT-3']

HWBR-F7 [5'-TTCCTCTGTGGTACCAAGAGGAATGC-3']

and HWBR-1910R:

[5'-GGTGGACTCGAGGTACCGATCTCACTCTCTTCCAGAATC-3']

PCR 1: One ng of pHHT13-1 DNA was used as template to do two PCRs with primers, HWBR-F7 vs HWBR/2BPI-506R and HWBR/2BPI-475F vs HWBR-1910R.

The Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842) was used under the following conditions:

5 µl of 10x Expand™ Buffer 1

7 µl of 2.5mM dNTP mix

1.5 µl of primer HWBR-F7 or HWBR/2BPI-475F

1.5 µl of primer HWBR/2BPI-506R or HWBR-1910R

0.75 µl of Expand PCR enzyme (1 unit)

33.25 µl water and

1 µl DNA.

Reaction conditions: 10 cycles of 92°C-40 sec; 48°C- 1 min; 68°C-3 min and 30 cycles of 92°C-40 sec; 55°C- 40 sec; 68°C-2 min.

The primers HWBR-F7 and HWBR/2BPI-506R amplified a DNA fragment of 400 bp and HWBR/2BPI-475F and HWBR-1910R amplified a DNA fragment of approximately 1.4 kb on an agarose gel. The two bands were cut out of the agarose gel and purified

with Qiaquick gel extraction kit from Qiagen Inc. (Cat no. 28706) and the DNAs were eluted in 50 µl of 10 mM Tris (pH 8.5).

PCR 2 (Extension without primers): Approximately 75 ng of two amplified product from above PCR 1 were mixed and then recombined without primers by extending under the following conditions:

2 µl of 10x Expand™ Buffer 1

2.8 µl of 2.5mM dNTP mix

0.3 µl of Expand PCR enzyme (1unit)

8.9 µl water

6 µl of combined PCR 1 products

Reaction conditions: 15 cycles of 92°C-1 min; 60°C- 5 min; 68°C-3 min.

PCR 3: 1 µl of amplified mix from PCR 2 was used as template to amplify with HWBR-F7 and HWBR- 1910R primers using the following conditions:

10 µl of 10x Expand™ Buffer 1

14 µl of 2.5mM dNTP mix

3.0 µl of primer HWBR-F7 or HWBR/2BPI-475F

3.0 µl of primer HWBR/2BPI-506R or HWBR-1910R

1.5 µl of Expand PCR enzyme (1 unit)

67.5 µl water and

1 µl DNA.

Reaction conditions: 30 cycles of 92°C-1 min; 60°C- 1 min; 68°C-2 min.

A DNA fragment of approximately 1.7 kb was amplified as seen on an agarose gel. The PCR product was purified using the QIAGEN's QIAquick PCR purification kit (cat. no. 28104) and eluted in 50 µl of 10 mM Tris, pH 8.0. The purified product was restricted with Kpn I and subcloned into Kpn I-restricted pcDNA3.1(-)/Myc-His A (Invitrogen, Cat. no. V855-20). One clone, named pc3.1/HuGL2R/MH6 (pHuMH6), had the 1.7 kb insert in correct orientation as checked by PCR using vector vs. insert primers.



Functional assay:

This hybrid clone was compared to rat GLP-2R using the assay described in Example 2. Results showed that the 2 bp "GA" replacement into the putative deletion site yielded a clone encoding a functional GLP-2R protein, as shown by the cAMP response to GLP-2 treatment.

#### EXAMPLE 6

##### Isolation of the Full-Length Human GLP-2 Receptor cDNA

Twenty thousand clones from  $\lambda$ gt10 cDNA Library from Human Stomach (Clontech; Cat. HL3017a) were plated on each of 100 agar 150 mm plates. SM buffer (0.1 M NaCl, 10 mM  $Mg_2SO_4$ , 35 mM Tris, pH-7.5, 0.01% gelatin) was added to each plate to obtain 100 phage lysates each containing 20,000 (20K) pooled clones. The first fifty 20K phage lysates (20K pools) were screened by PCR using two primers designed from HHT13 DNA sequence. The template DNA from each pool was prepared by boiling phage lysate for 10 minutes and centrifuging for 10 minutes.

HWBR-113F [5'-GTGGAGAGGATTTGTGCAAACATTTC-3']

HWBR-578R [5'-AGAGACATTTCAGGAGAAGAATGAG-3']

1  $\mu$ l of each 20K pool DNA was diagnosed by PCR with HWBR-113F and HWBR-578R primers using the following conditions:

2  $\mu$ l of 10x Expand<sup>TM</sup> Buffer 1

2.8  $\mu$ l of 2.5mM dNTP mix

0.6  $\mu$ l of primer HWBR-113F

0.6  $\mu$ l of primer HWBR-578R

0.3  $\mu$ l of Expand PCR enzyme (1 unit)

12.7  $\mu$ l water

1  $\mu$ l 20K pool DNA

Reaction conditions: 35 cycles of 92°C- 40s.; 60°C- 40s.; 68°C-1 min.

A DNA fragment of approximately 450 bp was seen in amplification of templates from two pools (HST 19 and HST 38).

B. Screening of clones from two positive pools: HST 19 and HST 38.

40,000 clones plated from each of two positive 20K pools were screened by plaque lifts on nitrocellulose filters (Amersham; Cat.RPN137E). The probe was prepared by random primer labeling a DNA fragment from pHHT13-1.

1. The filters were pre-hybridized and hybridized at 42°C overnight. Hybridization solution consisted of 50% formamide, 5X SSPE, 5X Denhart's solution, 0.5% SDS and salmon sperm DNA (200 mg/ml).

2. After hybridization the filters were washed under the following conditions:

- two times at room temperature in 2X SSPE and 0.01% SDS;
- two times at 42°C in 2X SSPE and 0.01% SDS; and
- two times at 50°C in 0.1X SSPE and 0.01% SDS.

3. The filters were autoradiographed and the regions on the plates matching to positive signals were isolated. One positive clone (HST 38-4-30) was isolated from HST 38 pool. 450 bp DNA fragment was amplified from the positive clone by using primers HWBR-113F and HWBR-578R and sequenced. The sequence clearly showed that the plasmid contain 2 bp (AG) at position 373-374 of HHT13 DNA sequence.

The complete insert of clone HST 38-4-30 was amplified using  $\lambda$ gt10 primers as described in Example 1. PCR amplified a DNA fragment of approximately 1.4 kb. The amplified DNA was purified and sequenced.

**EXAMPLE 4**

**Reconstruction of a clone of full-length functional human GLP-2R cDNA and functional assay**

A 700 bp fragment obtained by Kpn I and Pvu II restriction digest of the amplified DNA from clone HST 38-4-30, and 1.4 kb DNA fragment from Xho I and Pvu II restricted pHHT13-1 DNA were subcloned into Kpn I and Xho I restricted pcDNA3 in a three-way

ligation. The new plasmid construct was called pc3/HuGL2R-2. In this manner the full length sequence of the human GLP-2 receptor was obtained.

Functional Assay:

5 The new clone was compared to the rat GLP-2R clone 587 as described previously. Results showed that the clone encoded a functional human GLP-2R protein, which led to cAMP production in COS cells in response to GLP-2 treatment.

EQUIVALENTS

10 The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-described makes for carrying out the invention which are obvious to those skilled in the field of biochemistry, molecular biology or related fields are intended to be within the scope of the following claims.

15

What is claimed is:

1. An isolated nucleic acid encoding a GLP-2 receptor.

2. The isolated nucleic acid according to claim 1, wherein the nucleic acid encodes the amino acid sequence of SEQ ID NO: 2.

3. The isolated nucleic acid according to claim 2, having SEQ ID NO: 1.

4. The isolated nucleic acid according to claim 1, wherein the nucleic acid encodes at least the amino acid sequence of SEQ ID NO: 10.

5. The isolated nucleic acid according to claim 4, wherein the nucleic acid sequence comprises SEQ ID NO: 9.

6. An isolated nucleic acid having a sequence that is at least 40% homologous with SEQ ID NO: 1.

7. An isolated nucleic acid, characterized by:

(1) the ability to be amplified by PCR primers of SEQ ID NO: 3 and SEQ ID NO: 4; and

(2) the ability to bind a probe of SEQ ID NO: 5.

8. A recombinant polynucleotide molecule comprising a nucleic acid encoding a GLP-2 receptor, and expression controlling elements linked operably with said nucleic acid to drive expression thereof.

9. The recombinant polynucleotide molecule according to claim 8, adapted for expression in a mammalian cell.

10. The recombinant polynucleotide molecule according to claim 8, wherein said nucleic acid encodes a GLP-2 receptor of SEQ ID NO: 1.

11. The recombinant polynucleotide molecule according to claim 8, wherein said nucleic acid encodes a GLP-2 receptor comprising SEQ ID NO: 10.

12. A cell that has been genetically engineered by the insertion of nucleic acid coding for a GLP-2 receptor.

13. The cell according to claim 12, wherein said cell is adapted by insertion of a recombinant DNA molecule in which nucleic acid coding for an GLP-2 receptor and expression controlling elements functional in said cell are linked operably to drive expression of said nucleic acid.

14. The cell according to claim 13, wherein said nucleic acid encodes a GLP-2 receptor of SEQ ID NO: 1.

15. The cell according to claim 13, wherein said nucleic acid encodes at least the amino acid sequence of SEQ ID NO: 10.

16. The cell according to claim 14, wherein said nucleic acid is one having SEQ ID NO: 2.

17. A nucleic acid probe of at least 15 nucleotides, capable of specifically hybridizing with a unique region of a nucleic acid encoding a GLP-2 receptor.

18. An antisense oligonucleotide having a sequence capable of hybridizing to mRNA encoding a GLP-2 receptor so as to prevent translation of the mRNA.

19. An antibody which specifically recognizes a GLP-2 receptor.

08454880  
464240 9454880  
15  
20

20. A transgenic non-human animal, having incorporated expressibly therein a transgene which codes for a GLP-2 receptor.
- 5 21. A transgenic non-human animal comprising a homologous recombination knockout of DNA coding for the GLP-2 receptor native to said animal.
22. A transgenic non human animal which incorporates and expresses antisense DNA as defined in claim 18.
- 10 23. A recombinant GLP 2 receptor.
24. The GLP 2 receptor of claim 24, comprising SEQ ID NO: 1.
25. The GLP 2 receptor of claim 24, comprising SEQ ID NO: 10.
- 15 26. A method for identifying GLP 2 receptor ligands, which comprises the steps of  
(1) incubating a test ligand with a cell as defined in claim 9 or with a membrane preparation obtained therefrom; and then  
(2) determining the extent of binding between the GLP 2 receptor and the test ligand.
- 20 27. A method for identifying GLP 2 receptor ligands, which comprises the steps of  
(1) incubating a test ligand with a cell as defined in claim 10 or with a membrane preparation obtained therefrom; and then  
25 (2) determining the extent of binding between the GLP 2 receptor and the test ligand.
28. A GLP-2 receptor ligand identified by the method of claim 26.
- 30 29. An isolated nucleic acid according to claim 1, wherein the nucleic acid encodes a human GLP-2 receptor.

30. An isolated nucleic acid according to claim 29, wherein the nucleic acid encodes the amino acid sequence of SEQ ID NO: 12.

5 31. An antisense oligonucleotide according to claim 18, wherein said oligonucleotide has a sequence capable of hybridizing to mRNA encoding a human GLP-2 receptor.

32. An isolated nucleic acid having a sequence at least 40% homology with SEQ ID NO: 11.

08845546.042497

# ABSTRACT

The invention relates to nucleotides and amino acid sequences encoding glucagon-like peptide 2 receptors, recombinant host cells transformed with such nucleotides, and  
5 methods of using the same in drug screening and related applications.

08845546.042497



# DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

## CLONED GLUCAGON-LIKE PEPTIDE 2 RECEPTORS

and for which a patent application:

- ☒ is attached hereto and includes amendment(s) filed on \_\_\_\_\_ (if applicable)  
☐ was filed in the United States on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ (for declaration not accompanying application)  
with amendment(s) filed on \_\_\_\_\_ (if applicable)  
☐ was filed as PCT international application Serial No. \_\_\_\_\_ on \_\_\_\_\_ and was amended under PCT Article 19  
on \_\_\_\_\_ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
			YES <input type="checkbox"/> NO <input type="checkbox"/>
			YES <input type="checkbox"/> NO <input type="checkbox"/>

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED
08/767,224	December 13, 1996		X	

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Isaac Jarkovsky (Reg. No. 22713), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Fricbel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753), Jon R. Stark (Reg. No. 30111), Allan A. Famucci (Reg. No. 30256), Geraldine F. Baldwin (Reg. No. 31232), Victor N. Balancia (Reg. No. 31231), Albert P. Halluin (Reg. No. 25227), Samuel B. Abrams (Reg. No. 30605), Steven I. Wallach (Reg. No. 35402), Marcia H. Sundeen (Reg. No. 30893), Paul J. Zegger (Reg. No. 33821), Edmond R. Bannon (Reg. No. 32110), Bruce J. Barker (Reg. No. 33291), Adriane M. Amler (Reg. No. 32605), Ann L. Gisolfi (Reg. No. 31956), SaraLynn Mandel (Reg. No. 31853), Mark A. Farley (Reg. No. 33170), James G. Markey (Reg. No. 31636), and Charles F. Hoyng (Reg. No. 35548), all of Pennie & Edmonds LLP, whose addresses are 1155 Avenue of the Americas, New York, New York 10036, 1667 K Street N.W., Washington, DC 20006 and 2730 Sand Hill Road, Menlo Park, CA 94025, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO: PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, N.Y. 10036-2711				DIRECT TELEPHONE CALLS TO: PENNIE & EDMONDS DOCKETING (212) 790-2803	
201	FULL NAME OF INVENTOR	LAST NAME Munroe	FIRST NAME Donald	MIDDLE NAME E.	
	RESIDENCE & CITIZENSHIP	CITY Waterdown	STATE OR FOREIGN COUNTRY Ontario, Canada	COUNTRY OF CITIZENSHIP Canada	
	POST OFFICE ADDRESS	STREET 27 Wakefield Lane	CITY Waterdown	STATE OR COUNTRY Ontario, Canada	ZIP CODE L6R 2H3
202	FULL NAME OF INVENTOR	LAST NAME Gupta	FIRST NAME Ashwani	MIDDLE NAME K.	
	RESIDENCE & CITIZENSHIP	CITY Mississauga	STATE OR FOREIGN COUNTRY Ontario, Canada	COUNTRY OF CITIZENSHIP Canada	
	POST OFFICE ADDRESS	STREET 7031 Dunrobin Way	CITY Mississauga	STATE OR COUNTRY Ontario, Canada	ZIP CODE L5N 6Y4
203	FULL NAME OF INVENTOR	LAST NAME Vyas	FIRST NAME Tejal	MIDDLE NAME B.	
	RESIDENCE & CITIZENSHIP	CITY Mississauga	STATE OR FOREIGN COUNTRY Ontario, Canada	COUNTRY OF CITIZENSHIP Canada	
	POST OFFICE ADDRESS	STREET 275 Riel Drive	CITY Mississauga	STATE OR COUNTRY Ontario, Canada	ZIP CODE L5B 3K1
204	FULL NAME OF INVENTOR	LAST NAME McCallum	FIRST NAME Kirk	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Mississauga	STATE OR FOREIGN COUNTRY Ontario, Canada	COUNTRY OF CITIZENSHIP Canada	
	POST OFFICE ADDRESS	STREET 3618 Pitch Pine Crescent	CITY Mississauga	STATE OR COUNTRY Ontario, Canada	ZIP CODE L5L 1P8
205	FULL NAME OF INVENTOR	LAST NAME Fan	FIRST NAME Ermei	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Toronto	STATE OR FOREIGN COUNTRY Ontario, Canada	COUNTRY OF CITIZENSHIP Canada	
	POST OFFICE ADDRESS	STREET 20 Southport Street, Apt. #418	CITY Toronto	STATE OR COUNTRY Ontario, Canada	ZIP CODE M6S 4Y8
206	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 Donald E. Munroe	SIGNATURE OF INVENTOR 202 Ashwani K. Gupta	SIGNATURE OF INVENTOR 203 Tejal B. Vyas
DATE	DATE	DATE
SIGNATURE OF INVENTOR 204 Kirk McCallum	SIGNATURE OF INVENTOR 205 Ermei Fan	SIGNATURE OF INVENTOR 206
DATE	DATE	DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: ☒ Application of: Monroe, *et al.*  
☐ Patent of:

☒ Serial No.: To Be Assigned  
☐ Patent No.:

Group Art Unit: To Be Assigned

☒ Filed: Herewith  
☐ Issued:

Examiner: To Be Assigned

For: CLONED GLUCAGON-LIKE PEPTIDE 2  
RECEPTORS

Attorney Docket No.:  
8607-018-999

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
[37 CFR 1.9(f) and 1.27(c)] - Small Business Concern

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act in behalf of  
the concern identified below:

Name of concern Allelix Biopharmaceuticals Inc.

Address of concern 6850 Goreway Drive

Mississauga, Ontario, Canada L4V 1V7

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the person employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern and/or there is an obligation under contract or law by the inventor(s) to convey rights to the small business concern with regard to the invention, entitled CLONED GLUCAGON-LIKE PEPTIDE 2 RECEPTORS by inventor(s) Donald E. Munroe, Ashwani K. Gupta, Tejal B. Vyas, Kirk McCallum and Ermei Fan described in

- ☒ the specification filed herewith  
☐ application serial no. filed  
☐ patent no. issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_

☐ INDIVIDUAL      ☐ SMALL BUSINESS CONCERN      ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_

☐ INDIVIDUAL      ☐ SMALL BUSINESS CONCERN      ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_

☐ INDIVIDUAL      ☐ SMALL BUSINESS CONCERN      ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_

☐ INDIVIDUAL      ☐ SMALL BUSINESS CONCERN      ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28 (b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, or any patent to which this verified statement is directed.

Send correspondence to:      PENNIE & EDMONDS LLP      Direct Telephone calls to:  
1155 Avenue of the Americas      PENNIE & EDMONDS LLP  
New York, N.Y. 10036-2711      (212) 790-9090

Name of person signing Graham Strachan  
Title of person other than owner President and Chief Executive Officer  
Address of person signing Allelix Biopharmaceuticals Inc.  
6850 Goreway Drive, Mississauga, Ontario, Canada L4V 1V7  
Signature \_\_\_\_\_ Date \_\_\_\_\_

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

084546-04497

1 TCTCCACTCC CAACAGATGC GTCTGCTGTG GGGCCCTGGG AGGUCCTTCC  
51 TCGCCCTGCT TCTGCTGGTT TCCATCAAGC AAGTTACAGG ATCGCTCCTC  
101 AAGGAGACAA CTCAGAAGTG GGCTAATTAT AAGGAGAAGT GTCTGGAAGA  
151 CTTGCACAAT AGACTTTCTG GCATATTTTG TAATGGGACA TTTGATCGGT  
201 ATGTGTGCTG GCCTCATTCT TATCCTGGAA ATGTCTCTGT TCCCTGTCCT  
251 TCATACTTAC CTTGGTGGAA TGCAGAGAGC CCAGGAGGG CCTACAGACA  
301 CTGCTTGGCT CAGGGGACTT GGCAGACGCG AGAGAACACC ACAGATATTT  
351 GGCAGGATGA ATCAGAATGC TCAGAGAACC ACAGCTTCAG ACAAAACGTG  
401 GATCACTACG CCTTGCTATA CACCTTGCAg CTGATGTACA CTGTGGGCTA  
451 CTCCGTGTCT CTCATCTCCC TCTTCTTGGC TCTTACACTC TTCTTGTTC  
501 TTCGAAACT GCATTGCACA CGCAATTACA TCCACATGAA CCTGTTCGCT  
551 TCGTTCATCC TGAAAGTTCT GGCTGTCTTG GTGAGGACA TGGTCTCCCA  
601 CAACTCTTAC TCCAAGAGGC CCGATGATGA GAGTGGATGG ATGTCATATC  
651 TGTGAGAGAC ATCCGTCTCC TGTGCTCTCC TCCAGGTCCT CCTGCACTAC  
701 TTTGTGGGCA CCAATCACTT GTGGCTGCTG GTTGAAGGAC TTTACCTCCA  
751 CACTCTGCTG GAGCCCACAG TGTTTCCTGA AAGGCGGCTG TGGCCCAAGT  
801 ACCTGGTGGT GGGTTGGGCC TTCCCATGTC TGTTTGTAT TCCCTEGGGT  
851 TTTGCCCGTG CACACCTGGA GACACACGG TGCTGGGCCA CAAATGGGAA  
901 CCTGAAAATC TGGTGGATCA TCAGAGGACC CATGCTGCTT TGTGTACAG  
951 TTAATTTCTT CATCTTCCTC AAGATTCTCA AGCTTCTCAT TTCTAAGCTC  
1001 AAAGCTCATC AGATGTGCTT CAGAGACTAC AAATACAGAT TGGCGAATC  
1051 AACGTTGCTC CTCATTCTT TGTGGGGGT TCATGAGGTC CTCTTCACTT  
1101 TCTTCCCCGA CGACCAAGTT CAAGGATTTT CAAACGTAT TCGACTCTTC  
1151 ATCCAGCTGA CACTGAGCTC TGTCCACGGA TTTCTGGTGG CCTTGCACTA

FIGURE 1

1201 TGGCTTTGCC AATGGAGAGG TGAAGGCAGA GCTGCGAAG TCATGGGGCC  
1251 GCTTCTTATT AGCCCGCCAC TGGGGCTGCA GACCTGTGT CCTGGGGAAG  
1301 AATTTCCGGT TCCTGGGGAA GTGTTCCAAG AAGCTGTGG AGGGAGATGG  
1351 CTCTGAGACA CTCCAGAAGC TCGGTTCTC CACATGCAGC TCACACCTGG  
1401 CCTCTGAGAC CCTGGGAGAC GTTGGGGTAC AGCCTCACAG GGGCCGTGGA  
1451 GCTTGGCCCC GGGGAACCAG CCTGTCTGAG AGCAGTGAGG GAGACTTCAC  
1501 CCTGGCCAAT ACGATGGAGG AGATTCTGGA AGGAGTGAG ATCTAAGGCA  
1551 GGGTCCATCA CCGCAGCTTG GCCA

FIGURE 1A

08845546.04247

0884546-042497

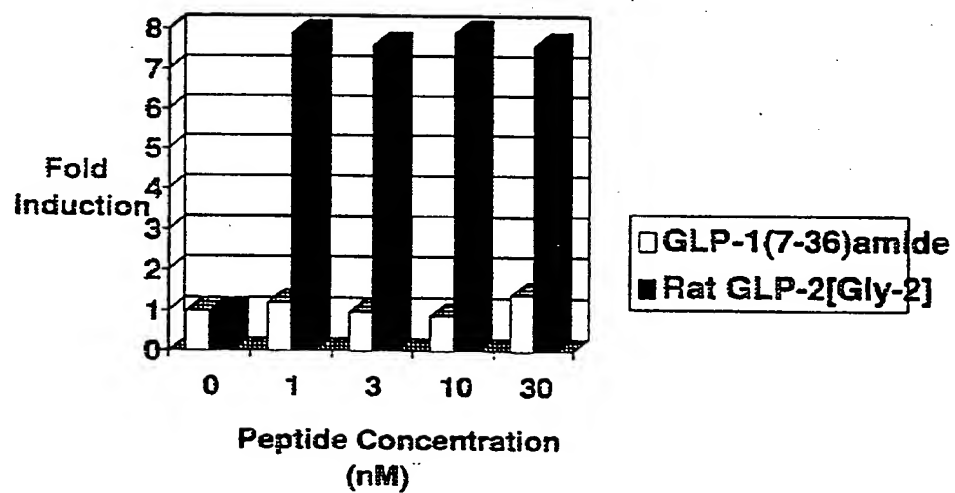
1 MRLWGPGRP FLALLLVSI KQVTGSLKE TTQWANYKE KCLEDLHRL  
51 SGIFCNGTFD RYVCWPHSYP GNVSVPCPSY LPWWNAESPG RAYRHCLAQG  
TM-1  
101 TWQTRENTTD IWQDESECSE NHSFRQNVDH YALLYTLOLM YTVGYSVSLI  
TM-2  
151 SLFLALTLEL FLRKLHCTR N YIHMNLEASF ILKVLAVLVK DMVSHNSYSK  
TM-3  
201 RPDDESGWMS YLSETSVSCR SVQVILHYFV GTNELWLLVE GLYLHTLLEP  
TM-4  
251 TVFPERRLWP KYLVVGWAFP MLFVIFWGP RAHLENTRCW ATNGNLKTIW  
TM-5 TM-6  
301 IIRGPMILCV TVNFFIFLKI IKLLISKLKA HQMCFRDYKY RLAKSTLLLI  
TM-7  
351 PLLGVHEVLF TFFEDDQVQG FSKRIRLFIO LTLSSVHGEL VALQYGFANG  
401 EVKAE LRKSW GRFLARHWG CRTCVLGKNF RFLGKCSKKL SEGDGSETLO  
451 KLRFTCSSH LASETLGDVG VQPHRGCAW PRGSSLSSESS EGDFTLANM  
501 EEILEESEI+

FIGURE 2

264240-9454880

Figure 3

cAMP Response of COS Transfected with Clone





HGLP2 Human GLP-2 Receptor C4-4 vs C9-2R PCR from Clone HMT13

1 TCCTTCTCTC TTATCTCCCT CTTCTGGCT CTCACCTCC TCTTGTTCT  
51 TCGAAACTC CACTGCACGC GCAACTACAT CCACATGAAC TTGTTTGCT  
101 CTTTCATCCT GAGAACCCTG GCTGTACTGG TGAAGGACGT CGTCTTCTAC  
151 AACTCTTACT CCAAGAGGCC TGACAATGAG AATGGGTGGA TGTCCTACCT  
201 GTCAGAGATG TCCACCTCCT GCCGCTCAGT CCAGGTTCTC TTGCATTACT  
251 TTGTGGGTGC CAATTACTTA TGGCTGCTGG TTGAAGGCCT CTACCTCCAC  
301 ACGCTGCTGG AGCCACAGT GCTTCCTGAG AGGCGGCTGT GGCCCARATA  
351 CCTGCTGTTG GGTGGGCCT TCCCTGTGCT ATTTGTTGTA CCCTGGGGTT  
401 TCGCCCGTGC ACACCTGGAR AACACAGGT GCTGGACAAC AAATGGGAAT  
451 AAGAAATCT GGTGGATCAT CCGAGGACCC ATGATGCTCT GTGTAACAGT  
501 CAATTTCTTC ATCTTCTGA AAATTCTCAA GCTTCTCATT TCTAAGCTCA  
551 AAGCTCATCA AATGTGCTTC AGAGATTATA AATACAGATT GGCAAAATCA  
601 AACTGGTCC TCATTCCTTT ATTGGGCGTT CATGAGATCC TCTTCTCTT  
651 CATCACTGAT GATCAAG

Figure 4

264240-9454880

264240-94554880

1	S F S L I S L F L A L T L L L F L R K L TCCTTCTCTTATCTCCCTCTCTGGCTCTCACCTCTCTTGTTCGAAACTC	60
61	H C T R N Y I H M N L F A S F I L R T L CACTGCACGGCAACTACATCCACATGAACCTGTTGCTTCTTCATCCTGAGAACCCTG	120
121	A V L V K D V V F Y N S Y S K R P D N E GCTGTACTGGTGAAGGACGTCTTCTACAACTCTTACTCCAAGAGGCTGACAATGAG	180
181	N G W M S Y L S E M S T S C R S V Q V L AATGGGTGGATGTCCTACCTGTCAGAGATGTCCACCTCTGCGGCTCAGTCCAGGTCTC	240
241	L H Y F V G A N Y L W L L V E G L Y L H TTGCATTACTTTGTGGGTGCCAATTACTTATGGCTGCTGGTTGAAGGCTCTACCTCCAC	300
301	T L L E P T V L P E R R L W P X Y L L L ACGCTGCTGGAGCCACAGTCTCTGAGAGGGCGCTGTGGCCARATACCTGCTGTTG	360
361	G W A F P V L F V V P W G F A R A H L E GGTTGGGCTTCCCTGTGCTATTGTGTACCTGGGGTTTCGCCCCGTGCACACCTGGAR	420
421	N T G C W T T N G N K K I W W I I R G P AACACAGGGTGCTGGACAACAATGGGAATAAGAAAATCTGGTGGATCATCCGAGGACCC	480
481	M M L C V T V N F F I F L K I L K L L I ATGATGCTCTGTGTAACAGTCAATTTCTTCATCTTCTGAAAATCTCAAGCTTCTCATT	540
541	S K L K A H Q M C F R D Y K Y R L A K S TCTAAGCTCAAAGCTCATCAATGTGCTTCAGAGATTATAAATACAGATTGGCAAAATCA	600
601	T L V L I P L L G V H E I L F S F I T D ACACTGGTCTCATTCCTTTATTGGGCGTTCATGAGATCCTCTTCTTCTTCATCACTGAT	660
661	D Q GATCAAG	667

Figure 5

(SEQ ID NO: 11)

1 TGGAGAGGATTGTGCAAACTTTCTTCTGTGGACCAAGAGGAATGCAAGAGGAGGCTGC 60  
CTGCGGTGCATCTTGGACGGCTAGAGAGATGTACCCCTACTTGTGAAGGTGCACGAGGAA 120  
61 M K L G S S R A G P G R G S A G L L P G  
GATGAAGCTGGGATCGAGCAGGGCAGGGCCTGGGAGAGGAAGCGCGGACTCCTGCCTGG 180  
121 V H E L P M G I P A P W G T S P L S F H  
CGTCCACGAGCTGCCATGGGCATCCCTGCCCCCTGGGGGACCAGTCTCTCTCTCTCCA 240  
181 R K C S L W A P G R P F L T L V L L V S  
CAGGAAGTGCTCTCTGGGCCCCCTGGGAGGCCCTTCTCACTCTGGTCTGCTGCTTTTC 300  
241 I K Q V T G S L L E E T T R K W A Q Y K  
CATCAAGCAAGTTACAGGATCCCTCCTTGAGGAACGACTCGGAAGTGGGCTCAGTACAA 360  
301 Q A C L R D L L K E P S G I F C N G T F  
ACAGGCATGTCTGAGAGACTTACTCAAGAACCTTCTGGCATATTTGTAAACGGGACATT 420  
361 D Q Y V C W P H S S P G N V S V P C P S  
TGATCAGTACGTGTGTGGCCCTCACTTCTCTCTGGAAATGTCTCTGTACCTGCCCCCTC 480  
421 Y L P W W S E E S S G R A Y R H C L A Q  
ATACTTACCTTGGTGGAGTGAAGAGAGCTCAGGAAGGGCCTACAGACACTGCTTGGCTCA 540  
481 G T W Q T I E N A T D I W Q D D S E C S  
GGGGACTTGGCAGACGATAGAGAACCACCGATATTTGGCAGGATGACTCCGAATGCTC 600  
541 E N H S F K Q N V D R Y A L L S T L Q L  
CGAGAACCACAGCTTCAAGCAAAACGTGGACCGTTATGCCTTGCTGTCAACCTTGACGT 660  
601 M Y T V G Y S P S L I S L F L A L T L L  
GATGTACACCGTGGGATACTCCTTCTCTTATCTCCCTCTCCTGGCTCTCACCCCTCCT 720  
661 L F L R K L H C T R N Y I H M N L F A S  
CTGTTTCTTGGAAAACCTCACTGCAACGCAACTACATCCACATGAACCTGTTTGCTTC 780  
721 F I L R T L A V L V K D V V F Y N S Y S  
TTTCATCCTGAGAACCCTGGCTGTACTGGTGAAGGACGTCGTCTTCTACAACTCTTACTC 840  
781 K R P D N E N G W M S Y L S E M S T S C  
CAAGAGGCTGACAATGAGAATGGGTGGATGTCCTACCTGTGAGAGATGTCCACCTCCTG 900  
841 R S V Q V L L H Y F V G A N Y L W L L V  
CCGCTCAGTCCAGTTCTCTTGCTTACTTTGTGGGTGCAATTAATGCTGCTGGT 960  
901 E G L Y L H T L L E P T V L P E R R L W  
TGAAGGCTCTACCTCCACACGCTGTGGAGCCACAGTGCTTCTGAGAGGCGGCTGTG 1020  
961

FIGURE 6A

084546-04497

1021	P R Y L L L G W A F P V L F V V P W G F GCCCAGATACCTGCTGTTGGGTTGGGCCTTCCCTGTGCTATTGTGTACCTGGGGTTT	1080
1081	A R A H L E N T G C W T T N G N K K I W CGCCCGTGACACCTGGAGAACACAGGGTCTGGACAACAAATGGGAATAAGAAAATCTG	1140
1141	W I I R G P M M L C V T V N F F I F L K GTGGATCATCCGAGGACCCATGATGCTCTGTGTAAACAGTCAATTTCTTCATCTTCCTGAA	1200
1201	I L K L L I S K L K A H Q M C F R D Y K AATTCTCAAGCTTCTCATTCTAAGCTCAAAGCTCATCAAATGTGCTTCAGAGATTATAA	1260
1261	Y R L A K S T L V L I P L L G V H E I L ATACAGATTGGCAAATCAACACTGGTCTCTCCTTTATTGGGCGTTCATGAGATCCT	1320
1321	F S F I T D D Q V E G F A K L I R L F I CTTCTCTTTCTACTGATGATCAAGTTGAAGGATTGCAAACCTTATACGACTTTTCAT	1380
1381	Q L T L S S F H G F L V A L Q Y G F A N TCAGTTGACACTGAGCTCCTTTTCATGGGTTCTGGTGGCCTTGCAAGTATGGTTTGCCAA	1440
1441	G E V K A E L R K Y W V R F L L A R H S TGGAGAAGTGAAGGCTGAGCTGCGGAAATACTGGGTCCGCTTCTGTAGCCCGCCACTC	1500
1501	G C R A C V L G K D F R F L G K C P K K AGGCTGCAGAGCCTGTGTCTCGGGAAGGACTTCCGGTTCTTAGGAAAATGTCCCAAGAA	1560
1561	L S E G D G A E K L R K L O P S L N S G GCTCTCGGAAGGAGATGGCGCTGAGAAGCTTCCGAAGCTGCAGCCCTCACTTAACAGTGG	1620
1621	R L L H L A M R G L G E L G A Q P Q O D GCGGCTCCTACATCTAGCCATGCGAGGTCTTGGGGAGCTGGGCGCCAGCCCCAACAGGA	1680
1681	H A R W P R G S S L S E C S E G D V T M CCATGCACGCTGGCCCCGGGGCAGCAGCCTGTCCGAGTGCAGTGAGGGGGATGTCACCAT	1740
1741	A N T M E E I L E E S E I * GGCCAAACACCATGGAGGAGATTCTGGAAGAGAGTGAGATCTAGGGTGGAGTTCACCACC	1800
1801	CTGGCTCTGCTCCAGGGACTCTTGAGGGGGCCAGGAAGAGGAAGCAAAGCAGGACACA	1860
1861	CGTTGCTGGGCACGGAATCATTCTCGTTCCATTCAACATGCCACTTTGATATGAAAGCTA	1920
1921	TCACAAGGTTCTTCAAGCTCTGTATGAAAGAGGCTGTGTGTCATGCTCACAGCCTCTGCC	1980
1981	TGCTCTTCTCATCCTAATAACCCCCACAGTGTGTTTTCCACAATGCCACCAGACCCCTA	2040
2041	GGGCCTGGCTCTAAATTCAAGCCAATGAAGTCCACCCGGAATTCTTTTGTCTTTTACCC	2100
2101	CTGGAAGAAATA	2112

FIGURE 6B

Human GLP-2 Receptor Complete Open Reading Frame  
Note: Translation may start with M-1 or M-26.  
Length: 553 April 21, 1997 07:42 Type: P Check: 2776 ..

1 MKLGSSRAGP GRGSAGLLPG VHELPMGIPA PWGTSPLSFH RKCSLWAPGR  
51 PFLTLVLLVŠ IKQVTGSLLE ETTRKWAQYK QACLRDLLKE PSGIPCNGTF  
101 DQYVCWPHSS PGNVSVPCPS YLPWSEESS GRAYRHCLAQ GTWQTIENAT  
151 DIWQDDSECS ENHSFKQNV DRYALLSTLQL MYTVGYSFSL ISLFLALTLL  
201 LFLRKLHCTR NYIHMNLFAS PILRTLAVLV KDVFVFNYSY KRPDNENGWM  
251 SYLSEMSTSC RSVQVLLHYF VGANYLWLLV EGYLHLLLE PTVLPERRLW  
301 PRYLLGWAF PVLFPVPWGF ARAHLENTGC WTTNGNKKIW WIIRGPMMLC  
351 VTVNPFIFLK ILKLLISKLE AHQMCFRDYK YRLAKSTLVL IPLLGVHEIL  
401 FSPITDDQVE GFAKLIRLFI QLTLSFPHGF LVALQYGFAN GEVKAELRKY  
451 WVRFLARHS GCRACVLGKD FRFLGKCPKK LSEGDGAELK RKLQPSLNSG  
501 RLLHLAMRGL GELGAQPOQD HARWPRGSSL SECSEGDVTM ANTMEEILEE  
551 SEI

264240" 94554880

FIGURE 7

264210-91554880

# cAMP Response to GLP-2

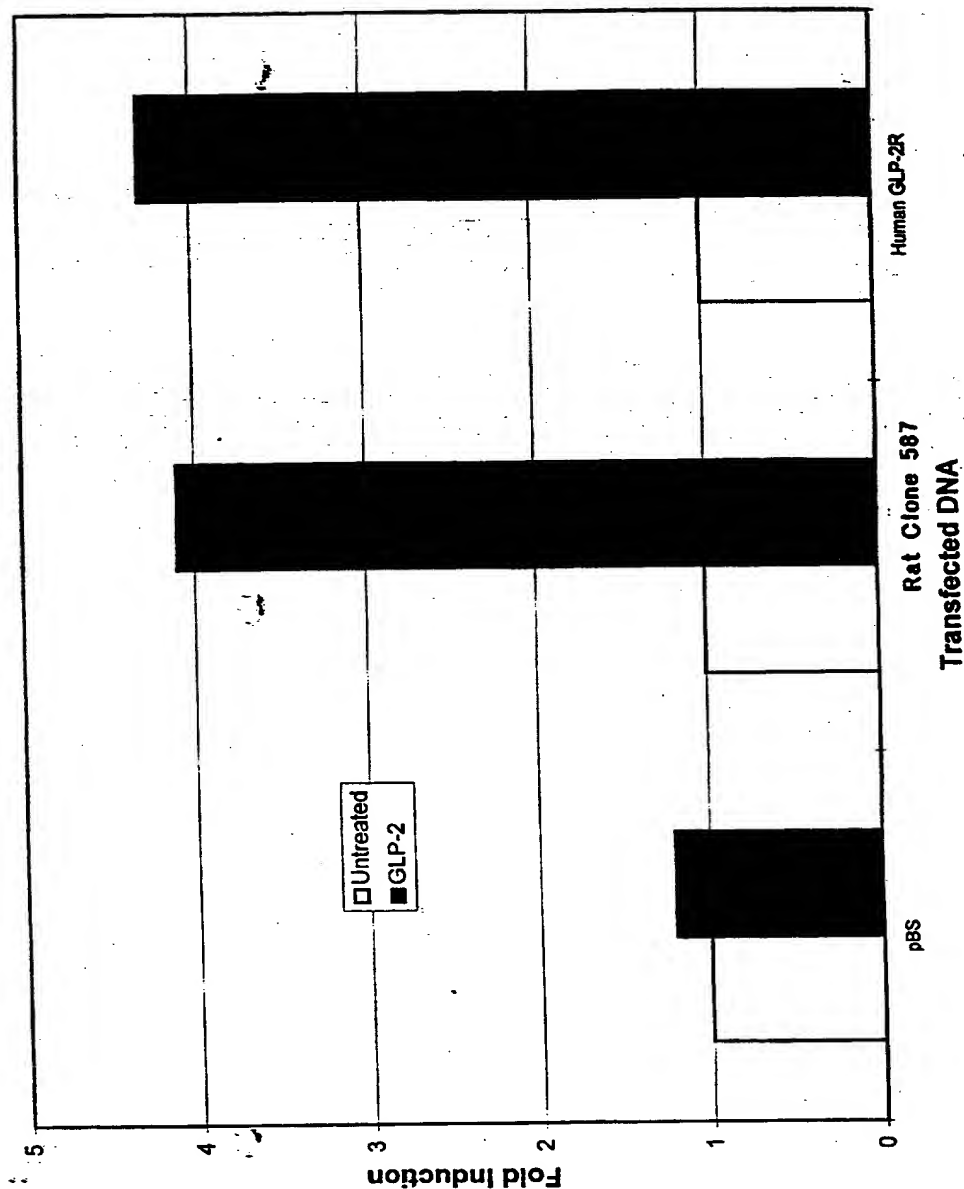


FIGURE 8

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

***This Page Blank (uspto)***